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Brain Serotonin Synthesis in MDMA (Ecstasy) Polydrug Users: An alpha-[¹¹C]Methyl-L-tryptophan Study

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Abbreviations used:

MDMA	3,4-Methylenedioxymethamphetamine
¹¹ C-AMT	Alpha-[¹¹ C]Methyl-L-Tryptophan
5-HT	5-Hydroxytryptamine, Serotonin

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US	United States
PET	Positron Emission Tomography
SERT	Serotonin transporter
K*	Blood-to-brain clearance/trapping
SCID-NP	Structured Clinical Interview for DSM-IV: Patient Edition
BDI	Beck Depression Inventory
CSF	Cerebrospinal fluid
5-HIAA	5-hydroxyindoleacetic acid
mCPP	meta-chlorophenylpiperazine
MR	Magnetic Resonance
MNI	Montreal Neurological Institute
FWHM	Full width at half maximum
SPM	Statistical Parametric Mapping
VOI	Volume of Interest
TPH	Tryptophan Hydroxylase
LSD	Lysergic acid diethylamide
L	Left
R	Right
BA	Brodmann area

Abstract

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) use may have long-term neurotoxic effects. In the present study, positron emission tomography with the tracer alpha-[^{11}C]Methyl-L-Tryptophan (^{11}C -AMT) was used to compare human brain serotonin (5-HT) synthesis capacity in 17 currently drug-free MDMA polydrug users with that in 18 healthy matched controls. Gender differences and associations between regional ^{11}C -AMT trapping and characteristics of MDMA use were also examined.

MDMA polydrug users exhibited lower normalized ^{11}C -AMT trapping in pre-frontal, orbitofrontal and parietal regions, relative to controls. These differences were more widespread in males than in females. Increased normalized ^{11}C -AMT trapping in MDMA users was also observed, mainly in the brain stem and in frontal and temporal areas. Normalized ^{11}C -AMT trapping in the brain stem and (pre)frontal regions correlated positively and negatively, respectively, with greater lifetime accumulated MDMA use, longer durations of MDMA use, and shorter time elapsed since the last MDMA use.

Although the possibility of pre-existing 5-HT alterations predisposing people to use MDMA cannot be ruled out, regionally decreased 5-HT synthesis capacity in the forebrain could be interpreted as neurotoxicity of MDMA on distal (frontal) brain regions. On the other hand, increased 5-HT synthesis capacity in the raphe and adjacent areas could be due to compensatory mechanisms.

Keywords: serotonin, Positron Emission Tomography, MDMA, ecstasy, drugs, tryptophan

Use of the drug 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) declined in the last decade in the United States (US), but consumption rates recently started rising again, possibly reflecting a reduced perception of risk (Johnston *et al.* 2012). The increase in prevalence may not be limited to the US, as there was also an increase in serious MDMA-related incidents in the Netherlands in 2011 (Krul *et al.* 2012). In 2010, the lifetime prevalence for MDMA use in the US was 13.5% for individuals between ages 19-30 (Johnston *et al.* 2011).

Although the incidence of death as a direct consequence of MDMA use is relatively low (Rogers *et al.* 2009), concerns remain about the drug's potential long-term harmful effects. Extensive animal studies demonstrate neurotoxicity following MDMA use, particularly in the 5-hydroxytryptamine (5-HT, serotonin) system. This includes distal axotomy in 5-HT neurons, reductions in 5-HT neuronal signaling, reduced regional density of the serotonin transporter (SERT) and lower activity of tryptophan hydroxylase (Capela *et al.* 2009), occasionally occurring after a single dose (Mueller *et al.* 2012).

Several studies have associated MDMA use with altered 5-HT function in humans. Positron Emission Tomography (PET) studies of MDMA users found evidence of altered *in-vivo* binding in cortical and/or sub-cortical areas, relative to controls, for tracers labeling brain SERT sites (see (Capela *et al.* 2009) for review). A recent PET study also reported that the recreational use of MDMA was associated with long-lasting increases in 5-HT_{2A} receptor density (Di Iorio *et al.* 2012).

Whether 5-HT alterations in MDMA users are pre-existing, due to MDMA or the other drugs used by MDMA users, or a combination of these, remain unknown. Nonetheless, given the important role of 5-HT in brain development, mood, behavior, and cognitive function (Parrott 2013), these alterations could have significant consequences for human functioning. Using Acute Tryptophan depletion, we recently reported that women who are polydrug MDMA users may be more susceptible than men to the effects of lowering 5-HT (Young *et al.* 2014). The present study focused on exploring further the likelihood of putative differences in 5-HT neurotransmission between MDMA polydrug users and controls, using an *in vivo* measure of regional 5-HT synthesis capacity with PET, in *real-time*.

In humans, brain regional 5-HT synthesis can be estimated *in vivo* with PET imaging of α -[^{11}C]Methyl-L-Tryptophan (^{11}C -AMT) as a tracer, using the blood-to-brain clearance/trapping (K^*) of the tracer (Diksic & Young 2001). The specificity and validity of the method has been demonstrated in both healthy and patient populations (Leyton *et al.* 2001, Leyton *et al.* 2005, Rosa-Neto *et al.* 2005, Lundquist *et al.* 2007, Rosa-Neto *et al.* 2007, Berney *et al.* 2008, Booij *et al.* 2010, Berney *et al.* 2011). In the present study, we used the ^{11}C -AMT method to compare brain 5-HT synthesis capacity in regular MDMA polydrug users who were free of drugs at the time of the scan, with non MDMA users. Our primary hypothesis was that polydrug users of MDMA would have “altered” regional ^{11}C -AMT trapping in cortical and/or subcortical areas, compared to age- and gender-matched individuals who had never used MDMA. Based on previous reports emphasizing differences in brain 5-HT synthesis between males and females (Nishizawa *et al.* 1997, Sakai *et al.* 2006), we also examined the regional K^* data in MDMA polydrug users for a putative gender effect. Finally, we examined whether ^{11}C -AMT trapping would be modulated by specific characteristics of MDMA use, such as an estimate of the cumulative lifetime MDMA dose, the duration of use and the time elapsed since the last MDMA dose.

Methods

Participants

Participants using MDMA, as well as MDMA-naïve controls, were recruited through newspaper advertisements, posters distributed at local universities and flyers distributed at local clubs and raves. Following a telephone interview to assess initial eligibility, participants underwent a full screening procedure in person involving the following: **(1)** A psychiatric assessment, including a semi-structured psychiatric interview (Structured Clinical Interview for DSM-IV: Patient Edition, SCID-NP) (First *et al.* 2002), the Beck Depression Inventory (BDI) (Beck *et al.* 1961), and a determination of the presence / absence of psychiatric illness in the family using the family history method (Andreasen *et al.* 1977). **(2)** A complete physical examination, including laboratory testing (glucose, electrolytes, alanine transaminase, aspartate aminotransferase, creatinine, thyroxine, thyroid- stimulating hormone, complete blood count) and an electrocardiogram. **(3)** Estimations of past and current drug use, using a timeline follow-back procedure as in our previous PET studies on drug use (e.g. (Boileau *et al.* 2006)).

The main inclusion criterion for MDMA polydrug users was the use of MDMA on at least 25 occasions, a level of use which has been previously associated with evidence of brain alterations in the brain serotonergic system such as altered cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) levels, altered brain SERT binding and altered mood and neuroendocrine responses to the 5-HT agonist probe meta-chlorophenylpiperazine (mCPP) (McCann *et al.* 1999). These criteria were similar to those used in a previous study on MDMA polydrug users conducted in our lab, using Acute Tryptophan Depletion (Young *et al.* 2014). Exclusion criteria included **(1)** a current or past major medical illness (determined by physical examination and laboratory tests), **(2)** evidence for current axis I DSM-IV disorders, **(3)** current use of any prescription psychotropic drug, **(4)** a BDI score of 12 or above, **(5)** reports suggesting evidence of a positive family history in first-degree relatives for Major Depressive Disorder, and **(6)** being a sexually active woman and not using a reliable form of contraception.

Assessment of the controls was similar to that of the MDMA polydrug users. Potential control participants reporting some illegal drug use other than MDMA were not in principle excluded from the study; selecting controls reporting no drug use could have resulted in a control cohort that would be too different from that of our MDMA users. Participants with less than five

total uses, and no use in the previous year, of illicit drugs other than cannabis, were deemed eligible. For cannabis, we only included controls, if their use in the previous year averaged less than once a month.

Participants were asked to refrain from drug use for any drug, three weeks prior to the study sessions. They were required to test negative on two consecutive urine drug tests for the detection of illicit drugs, one at screening and one on the morning of the PET session (Triage, San Diego, USA). All participants who were suitable and willing to give informed consent were scheduled. Once a full description of the study had been completed, written informed consent from all participants was obtained. The study was in conformance with the code of ethics of the World Medical Association. The Research Ethics Boards of the McGill University Health Centre and the Montreal Neurological Institute (MNI) approved the study.

Positron Emission Tomography (PET) and Magnetic Resonance (MR) Imaging

All participants underwent a 60-minute dynamic PET scan, conducted with an ECAT HR+ (CTI Molecular Imaging, Inc/Siemens, Knoxville, Tennessee) in the late morning or early afternoon. The participants received a low-protein diet the previous day and fasted overnight (limited water intake was allowed). Women underwent the PET scan in the follicular phase of their menstrual cycle. Two intravenous lines were secured, one in each arm, with one used for tracer injection and one for blood withdrawal. Before beginning the dynamic PET scan, transmission scans were performed using a ^{68}Ga source for attenuation correction. Participants received approximately 10 mCi of ^{11}C -AMT over 2 minutes, at the beginning of the dynamic PET scan protocol. Twenty-six frames were acquired using the following protocol: 6 frames of 30 seconds, 7 frames of 60 seconds, 5 frames of 120 seconds and 8 frames of 300 seconds. All scanning was acquired with a Neuro-Shield® placed around the participant's neck in order to reduce scattering. Blood samples were drawn throughout the PET scan from the ante-cubital vein at progressively increasing time intervals in order to obtain the ^{11}C -AMT plasma time activity curves. The input function was estimated using a validated non-invasive procedure combining venous sinus activity (first 20 min) and venous plasma time-activity data (Nishizawa *et al.* 1998, Nishizawa *et al.* 1997). Specifically, radioactivity-time courses from the venous samples were drawn, and corrected by the venous sinus from dynamic PET images, as reported in Nishizawa *et al.* (1998). The slopes K^* were then calculated using normalized exposure time points (Nishizawa *et al.* 1998).

PET scans included 63 image slices at an intrinsic resolution of 5.0 x 5.0 x 5.0 mm full width at half maximum (FWHM). Images were reconstructed using a Hanning filter of 8.1 mm FWHM in the trans-axial direction.

All participants also underwent high-resolution MR imaging using a 1.5-T system (Philips Gyro-scan; Philips Medical Systems, Eindhoven, the Netherlands) for the purpose of PET/ MRI co-registration. MR imaging data were stored as 256 x 256 x 160 mm matrices with 1-mm³ isotropic voxels.

Tryptophan measurement

Three 2-ml venous blood samples were drawn from each individual during the PET scan. The samples were centrifuged, and the plasma was stored at -80°C for measurement of plasma free tryptophan concentration using high-performance liquid chromatography (Nishizawa *et al.* 1997).

Statistical Analyses

Differences in demographics, drug use, plasma tryptophan values and behavior between the MDMA polydrug users and controls were tested using one-way ANOVA or chi-square statistics in the case of nominal variables.

Both the co-registration and normalisation were conducted according to standardized procedures used at the MNI (e.g. (Rosa-Neto *et al.* 2004, Leyton *et al.* 2001, Booij *et al.* 2010, Berney *et al.* 2011, Booij *et al.* 2012). Briefly, co-registration of the individual PET and MRI images was performed using an automatic procedure (Woods *et al.* 1993), which uses averaged tissue activity images obtained during the time period of 5 to 60 minute of dynamic PET data acquisition (Okazawa & Diksic 1998). Parametric K* images of ¹¹C-AMT trapping were generated (Okazawa & Diksic 1998) and re-sampled into MNI305 2-mm isotropic stereotaxic space using a standard automatic algorithm (Collins *et al.* 1994). The images were subsequently smoothed to a 14-millimeter resolution FWHM, using an isotropic Gaussian filter. In order to cancel out the effects of individual global effects on regional ¹¹C-AMT trapping (K*) values, statistical analyses with proportional scaling was used and regional K* values were normalized by the mean global K* of the gray matter to 100, as in our previous studies (Rosa-Neto *et al.*

2004, Leyton *et al.* 2001, Booij *et al.* 2010, Berney *et al.* 2011, Booij *et al.* 2012). The rationale for normalizing to the mean global K^* of the grey matter is to remove changes due to brain size, higher blood flow, etc. within and between groups. The number 100 was chosen in order to express normalized K^* in percentages.

A Student's t-test was applied voxel-by-voxel, to determine regional differences in the K^* distribution pattern between MDMA polydrug users and controls. Statistical significance was defined by two criteria. First, the height threshold used to interpret the t-test in terms of probability level, was set at $p=0.005$, thereby, accounting for multiple testing, while keeping the chance of a type II error under control. Second, the extent threshold was set at 40 voxels.

Further analyses examined regional K^* for gender differences by conducting separate analyses for men and women and tested associations with specific characteristics of MDMA polydrug use.

All of these analyses were carried out in Statistical Parametric Mapping (SPM) version 8 (SPM8; Wellcome Functional Imaging Laboratory, London, United Kingdom). In order to confirm and quantify the results obtained from whole brain SPM analyses, Volumes of interest (VOI) analyses were performed in those specific brain regions that showed significant group differences in the whole brain SPM analyses and were large enough to be reliably identified on each participant's MRI using an automatic segmentation method (Collins *et al.* 1999). Regional K^* was expressed both as normalized and non-normalized values; the need to normalize the regional K^* data in the present study was supported by the observed trend in gender differences in the control group in plasma free tryptophan values (see below). Group differences in normalized K^* extracted from the VOI analyses were analyzed using general linear models (GLM) for repeated measures, with hemisphere as a within subject factor, and group (MDMA polydrug user vs. control) as between subjects factor. Significant group by hemisphere interactions were further investigated using one-way GLM models, using the left and right hemisphere for the specific VOI investigated, as a dependent variable, and group, as a between subject factor. One-way GLM models, without a hemisphere term, were run for the analyses of the raphe and brain stem given that these are very small regions. As was the case for

whole brain SPM analyses, all GLM analyses were rerun for males and females separately. The magnitude of group differences in normalized regional K^* identified by VOI analyses was further quantified by calculating Cohen's d effect sizes using the G-power program (Faul *et al.* 2007).

Participants

Sample characteristics are shown in Table 1. Self-reported MDMA consumption varied significantly from 28 to 1015 tablets over the subject's lifetime (mean $\pm$ SD: 236 ± 282 ; median: 100), over periods of time ranging from 1 to 9 years (4 ± 2.3 years; median: 3.5). Compared to other samples reported in the literature, this corresponds to a "moderate" to a "significant" pattern of use. The controls all reported never having used MDMA. Consistent with what is normally observed in the general population, all MDMA users reported using other drugs as well (Table 2). There were no gender differences in reported lifetime MDMA intake, duration of MDMA use or time elapsed since the last MDMA dose, nor in the lifetime consumption of alcohol or of any other drugs. All participants were free of drugs when participating in the study, as verified by the urine drug tests obtained during screening and on the morning soon prior to the PET measurement.

Plasma tryptophan

The mean plasma free tryptophan concentrations $\pm$ SD were 9.5 ± 2.3 nmol/ml for controls and 10.3 ± 2.3 nmol/ml for MDMA polydrug users (Table 1). This group difference was not significant ($t(32)=0.91$, $p=0.37$). There was no gender difference (mean $\pm$ SD: 9.4 ± 2.5 nmol/ml for males and 10.4 ± 2.6 nmol/ml for females, $t(32) = -1.2$, $p=0.24$), although in controls, there was a trend towards higher plasma free tryptophan levels in females than in males (control males: 8.3 ± 2.0 nmol/ml; control females: 10.7 ± 3.2 nmol/ml) ($t(16)=-1.92$, $p=0.073$).

^{11}C -AMT trapping in MDMA polydrug users vs. controls

Whole brain SPM analyses. MDMA polydrug users demonstrated focal areas of both increased and decreased regional normalized K^* values, relative to controls ($p<0.005$, clusters ≥ 40) (Figure 1). MDMA polydrug users had decreased ^{11}C -AMT trapping extending from the pre-frontal orbital-frontal regions all the way to posterior parietal regions. Notably, the main focus of increased uptake in MDMA polydrug users was in the brain stem, in the region of the

periaqueductal gray matter, as well as in parts of the left lateral prefrontal cortex and temporal lobe (**Table 3**).

VOI analyses. MDMA polydrug users had, relative to controls, higher regional normalized K^* in the superior temporal gyrus ($F(1,33)=9.54$, $p=0.004$, $d=1.06$) and middle frontal gyrus ($F(1,33)=5.08$, $p=0.03$, $d=0.77$) in both hemispheres. Trends of increased normalized K^* in MDMA polydrug users relative to controls, were also observed across the brain stem ($F(1,33)=3.58$, $p=0.07$, $d=0.65$) and for the raphe specifically ($F(1,33)=2.94$, $p=0.096$, $d=0.71$). There were significant group by hemisphere interactions for the superior frontal gyrus ($F(1,33)=4.53$, $p=0.04$). Compared to controls, MDMA polydrug users had higher normalized K^* in the left superior frontal gyrus (relative to the right superior frontal gyrus) and lower normalized K^* in the right precuneus (relative to the left precuneus), although not to a statically significant degree when analyzing hemispheres separately ($d=0.60$ and $d=0.61$, respectively).

Gender effects of MDMA polydrug use on ^{11}C -AMT trapping

Whole brain SPM analyses. A separate whole brain analyses for males and females indicated that lower normalized ^{11}C -AMT in the frontal regions was especially noticeable in males (**Table 4**). Increases in normalized ^{11}C -AMT trapping in the brain stem and parts of the frontal and temporal lobe as observed in the entire group of MDMA polydrug users were not observed when analyzing males and females separately. Sample sizes were insufficient to reliably examine correlations between specific characteristics of MDMA use and ^{11}C -AMT trapping for males and females separately.

VOI analyses (Table 5). Overall, VOI analyses were largely consistent with results drawn for whole brain analyses. Male MDMA polydrug users had lower normalized K^* in the precentral gyrus, relative to control males ($F(1,16)=5.73$, $p=0.029$, $d=1.14$). Group by hemisphere interactions were observed in the precentral gyrus ($F(1,16)=5.79$, $p=0.029$) and in the precuneus ($F(1,16)=9.26$, $p=0.008$). Univariate one-way GLM analyses for the left and right hemispheres for each of these regions indicated that male MDMA polydrug users had lower normalized K^* for both the pre central gyrus and precuneus in the right hemisphere ($d=1.47$ and $d=1.10$, respectively), relative to male controls. Female MDMA polydrug users had lower normalized K^*

in the lateral orbitofrontal gyrus ($F(1,15)=5.45$, $p=0.03$, $d=1.14$), relative to female controls. In addition, increased normalized K^* in the raphe observed in the MDMA polydrug users, was mainly due to an increased normalized K^* in this region in female MDMA polydrug users ($F(1,15)=8.55$, $p=0.01$, $d=1.43$).

Correlations between ^{11}C -AMT trapping and characteristics of MDMA use (Fig 2)

A greater lifetime MDMA intake was associated with decreased ^{11}C AMT trapping in a very small frontal cortical area in the right hemisphere, although only at a marginally significant level (T peak=3.38, k max=41). A greater lifetime MDMA intake was also associated with higher normalized ^{11}C -AMT trapping in clusters of voxels projecting in the raphe area or in close approximation to it (anterior and superior right cerebellar hemisphere adjacent to the brain stem) (T peak=3.48, k max=28). There was no significant correlation between the total dose of any of the other drugs of abuse and normalized ^{11}C AMT trapping.

A longer duration of MDMA use was associated with lower normalized ^{11}C -AMT trapping in anterior regions of the brain, particularly in the mid-portion of the cingulate gyrus (Peak $T=5.02$, k max=933). Duration of MDMA use correlated positively with ^{11}C -AMT trapping in cortical regions covering mostly the postcentral regions of the brain (peak $T=5.29$, k max=201). The latter correlation was confirmed by VOI analyses for the left postcentral gyrus ($r=0.695$, $p=0.002$).

A shorter MDMA-free time interval, defined as the time elapsed from the last MDMA dose and the date of the PET scan, was associated with lower ^{11}C -AMT trapping in a few regions in the right hemisphere neo cortex (peak $T=4.07$, k max=93) as well as with higher ^{11}C -AMT trapping in the right cerebellar hemisphere (peak $T=5.73$, k max=270) and the brain stem (including the raphe region). The latter association was confirmed by VOI analyses ($r=-0.51$, $p=0.05$).

Discussion

This study points to regional differences between MDMA polydrug users and matched controls for 5-HT synthesis capacity, with both increases and decreases in ^{11}C -AMT trapping in MDMA polydrug users relative to controls. While decreases were primarily observed in prefrontal-orbital and parietal regions, with somewhat more widespread decreases in males relative to females, increases in ^{11}C -AMT trapping was also noticeable, in particular in the brain stem.

Our results are consistent with studies in rodents and non-human primates indicating that repeated administration of MDMA often results in long-term region-specific reduction of numerous 5-HT markers, including brain tissue concentrations of 5-HT, 5-HIAA levels, Tryptophan Hydroxylase (TPH) enzyme activity and SERT density (Urban *et al.* 2012, Biezonski & Meyer 2011). These reductions in 5-HT markers have previously been interpreted as a result of distal axotomy of brain 5-HT neurons (Molliver *et al.* 1990). However, it is noteworthy that in our study, the spatial extent of focal declines in cingulate cortex and mesencephalon is less than might have been expected on the basis of SERT imaging studies in similar populations or in preclinical studies where a loss of small serotonin fibers in the forebrain (see also below) was exhibited as a result of MDMA. In addition to decreases in ^{11}C -AMT trapping in the frontal regions, increased normalized ^{11}C -AMT trapping was also observed, mainly in the brain stem as well as in the superior medial and temporal gyri.

Using the same tracer and radio-autography 14 days after rats were administered a total of 8 doses of MDMA, there were significant decreases in 5-HT in a variety of brain areas including parts of the cortex, striatum and hippocampus, and a significant increase only in the median raphe nucleus (Molliver *et al.* 1990). This suggests that, in addition to MDMA-induced neurotoxicity in humans, compensatory feedback mechanisms in the cell bodies of the 5-HT neurons might also occur. This observation was consistent with findings from a longitudinal study in MDMA polydrug users with repeated measurements of brain SERT binding, which reported increases in SERT binding after cessation of MDMA use (Buchert *et al.* 2006). Indeed, a large animal literature reports on the extent to which the effects of MDMA may be enduring / persistent, as opposed to reversible. For instance, although studies in rats have shown that there is almost complete recovery of 5-HT markers one year after drug exposure, as a result of

sprouting of axons (Molliver *et al.* 1990), studies in monkeys reported that the recovery is incomplete, and innervation patterns remain altered (Hatzidimitriou *et al.* 1999, Scheffel *et al.* 1998). In squirrel monkeys that had been treated with MDMA and monitored for up to 7 years thereafter, some of the 5-HT “deficits” “recorded” at the end of the study were actually less severe than those observed at 18 months following exposure. Factors influencing recovery included the distance from the raphe nuclei to the purported affected site, the degree of initial axonal injury, and possibly the proximity of myelinated fibers (Hatzidimitriou *et al.* 1999). PET studies of SERT distribution in baboons also suggested that there is some recovery in regions closer to the raphe nuclei. Specifically, at 13 months after MDMA exposure, SERT levels had actually risen above control levels in the hypothalamus, but were still down respectively by 62, 78 and 73% in the frontal, parietal and occipital cortex (Scheffel *et al.* 1998). A more recent autoradiography study in rats investigating the impact of MDMA on 5-HT biosynthesis found that 14 days after chronic MDMA administration, TPH₂ enzyme immuno-reactivity was decreased, while TPH₂ mRNA was increased in the cell bodies of 5-HT neurons in the Dorsal Raphe Nucleus, suggesting some compensatory mechanisms (Bonkale & Austin 2008).

The impact of polydrug use on brain 5-HT synthesis appeared to be more widespread in males than in females. Interestingly, there were no differences in reported lifetime MDMA intake, duration of MDMA use or time elapsed since the last MDMA dose, nor in the lifetime estimate of alcohol or of other drugs examined consumed. At first glance, these results seemed to be at odds with findings of previous studies, showing that women appear to be more susceptible to the neurochemical effects of MDMA on CSF 5-HIAA (McCann *et al.* 1994) and SERT binding (Reneman *et al.* 2001), than men. They are also at first glance at odds with our recent ATD study, indicating a greater mood-lowering response to ATD in female MDMA polydrug users than in males (Young *et al.* 2014). Yet, previous studies in animals did not find compelling evidence of gender effects of MDMA on the 5-HT system (5-HIAA or 5-HT concentrations) (Walker *et al.* 2007). Methodological differences across studies, genetic differences, polydrug use, hormonal factors, and MDMA dose might account for some of those different observations. On the other hand, in spite of the observation that the effects of MDMA polydrug use in males may be more widespread than in females, interestingly, female MDMA polydrug users in the present study had, relative to female controls, a relatively specific lowering in regional K* in the

orbitofrontal regions, a brain region highly involved in emotion regulation. Alterations in regional K* in the orbitofrontal regions were absent in male polydrug users. Previous PET studies assessing cerebral metabolism following ATD have shown that the mood-lowering response to ATD in depression vulnerable populations correlates with alterations in metabolism in the orbitofrontal regions after ATD (Bremner *et al.* 1997, Smith *et al.* 1999, Neumeister *et al.* 2004). Taking the findings of our ATD study and from the current PET study together, it might be that the mood-lowering effect of ATD in female polydrug users as observed in (Young *et al.* 2014) are due to sex-specific, drug induced, 5-HT effects in the orbitofrontal regions. Only four female MDMA polydrug users participated in both the ATD study and the current PET study and thus correlations between the mood response to ATD and 5-HT synthesis capacity could not be reliably tested; yet, it is tempting to speculate that sex-specific effects of drug use on 5-HT neurotransmission in the orbitofrontal regions might make female users more susceptible to aversive behavioral manifestations, including clinical depression. Longitudinal studies in (former) drug users having consumed MDMA in significant quantities over long periods of time are needed to test such hypothesis.

Strengths and limitations of this study and suggestions for future research

The validity and significance of the observations presented in this study rest upon the following considerations: **(1)** Despite the criticism that the brain regional uptake of ¹¹C-AMT reflects blood-brain transport of tryptophan, rather than 5-HT synthesis (Shoaf *et al.* 1998), experimental evidence accumulated over more than 15 years by us and other research groups (Leyton *et al.* 2005, Lundquist *et al.* 2007), now provides a firm basis for the claim that brain regional ¹¹C-AMT trapping represents an acceptable proxy for the measurement of regional brain 5-HT synthesis. **(2)** As is the case for other MDMA PET studies, we cannot rule out the possibility that some of the alterations reported could be pre-existing and related to characteristics particular to individuals who use MDMA. **(3)** In addition to MDMA, many other drugs have been shown to influence 5-HT neurons, and the combination of MDMA and such drugs that are often used together with MDMA, e.g. methamphetamine, could have a greater effect on brain monoamines systems than either compound alone (Yuki *et al.* 2013). Thus, the group differences in 5-HT synthesis capacity may be due to the use of other drugs, either alone or in combination with MDMA. However, the fact that our results are consistent with those reported in rats administered

MDMA (Muck-Seler *et al.* 1998) and studied with a similar technique, supports the general view that the effects depicted in this study are primarily due to MDMA. This interpretation is further strengthened by the observation that the pattern of effects on 5-HT systems observed in the present study, was different from what was observed after administration of alcohol, nicotine, or cannabis (Jang *et al.* 2002, Nishikawa *et al.* 2009, Shen *et al.* 2011). **(4)** Though our sample size was comparable with other PET studies in MDMA polydrug users, the results were not significant when corrected for Family-Wise Error. However, most of the regions exceeded largely the a-priori determined and commonly used threshold criteria, (mostly $K > 100$ and $p < 0.001$) and the VOI analyses generally did not contradict the results obtained with SPM, thereby controlling for type I errors. **(5)** Alterations in the midbrain of brain 5-HT synthesis, could have occurred in many different ways, unrelated to MDMA use. **(6)** In our initial study, healthy females had overall a 52% lower 5-HT synthesis rates than healthy men (Nishizawa *et al.* 1997). The observation of lower 5-HT synthesis capacity in healthy females relative to healthy males have been replicated in a later study, using normalized regional K^* data (Sakai *et al.* 2006), although these effects were region and hemisphere specific (Sakai *et al.* 2006); an observation we also noticed in the present study, albeit to a non-significant degree. Our control sample was twice as smaller than Sakai *et al.* (2006) suggesting that the present study was underpowered to study reliably gender differences in regional K^* . However, the influence of other factors, recently shown to be associated with subtle variation in brain 5-HT synthesis in carefully screened healthy populations, cannot be ruled out, including (but not limited to) genetic variation (Booij *et al.* 2012). Both males and females showed increases in normalized K^* in the brain stem. **(7)** Male MDMA polydrug users tended to have increases in regional K^* across the brain stem, relative to male controls, while female MDMA polydrug users showed significant alterations specifically in the raphe. Indeed, even in a small brain structure such as the raphe, the effects of MDMA appear to vary within specific subregions (Muck-Seler *et al.* 1998).

Taken together, the data reported both in this and one recently published manuscript by our group (Young *et al.* 2014), examining in MDMA polydrug users the functional behavioral and/or neurochemical consequences of MDMA use, allow for the following observations: **(1)** Previous studies in humans assessing aspects of 5-HT neurotransmission in vivo, including while using 5-HT-related radio-ligands, combined with PET, have generally reported *decreased* 5-HT function

in MDMA polydrug users, relative to controls. Some evidence is provided here that this observation can be further extended to brain 5-HT synthesis. Together with findings obtained from animal studies (Muck-Seler *et al.* 1998), the results also provide some evidence that MDMA has a possible neurotoxic effect on 5-HT fibers in brain regions involved in emotion-regulation. (2) The “neurotoxic” effects of MDMA appear to be different for males and females. Although the effects appear to be regionally more widespread in males across the brain, deleterious effects on 5-HT synthesis may occur in specific orbitofrontal regions in females (3) The “neurotoxic” effects of MDMA, indicated by the observation of a *decreased* regional 5HT synthesis capacity, may coexist with evidence of *increased* activity in other sites, suggesting some capacity for regeneration of 5-HT systems. Unfortunately, this comment is only speculative, since our study was cross-sectional and not longitudinal.

Conclusions

In summary, the present study provides evidence that MDMA polydrug users have regionally specific increases and decreases in brain 5-HT synthesis relative to controls. MDMA-associated decreases in 5-HT synthesis in the frontal regions could be interpreted as MDMA-induced axotomy effects, while increases in the brain stem might be explained by compensatory changes in cell bodies.

Acknowledgements. / Conflict of Interest disclosure

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Figure legends

Fig 1. Normalized ^{11}C -AMT trapping in MDMA polydrug users vs. controls. The upper three color coded images indicate brain regions in which there is lower ^{11}C -AMT trapping in MDMA polydrug users than in controls (MDMA users < controls). The lower three color coded images indicate brain regions in which there is higher ^{11}C -AMT trapping in MDMA polydrug users than in controls (MDMA polydrug users > controls).

Fig 2. Correlation between ^{11}C -AMT trapping and indices of drug use in MDMA polydrug users. The color-coded images represent the strength of correlations between ^{11}C -AMT trapping and lifetime MDMA use (upper row), duration of MDMA use (middle row) and time elapsed since last MDMA use (bottom row). The first three images in each row (left side) depict positive associations with ^{11}C -AMT trapping, the last three images in each row (right side) depict negative associations.

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Table 1 Characteristics of the sample

Characteristic	Controls			MDMA polydrug users		
	N			N		
Gender	9 males, 9 females			9 males, 8 females		
	Mean	SD	Range	Mean	SD	Range
Age (years)	22.2	3.1	19-29	23.3	3.0	19-29
BDI score	2.3	2.6	0-9	3.9	3.2	0-10
Plasma free tryptophan (nmol/L)*	9.5	2.8	5.3-17.8	10.3	2.3	6.5-15
Activity injected (mCI)	9.3	1.1	7.1-10.5	9.8	0.4	9.3-10.5
Number of MDMA tablets, lifetime	0	n/a	n/a	236	282	28-1015
Estimated age of first MDMA use	n/a	n/a	n/a	19.2	2.8	15-24
Time since last MDMA dose in weeks	n/a	n/a	n/a	11.6	10.5	1-33
Duration of use in years	n/a	n/a	n/a	4.0	2.3	1-9

^a Significant difference ($p=0.03$) from value for control group (one-way ANOVA).

BDI= Beck Depression Inventory. n/a = not applicable. Difference in MDMA use cannot be tested statistically because both mean and SD in control group is 0. One value (female control) was missing and thus not included in the analyses.

Table 2 Self-reported lifetime drug/alcohol consumption other than MDMA, expressed as number of estimated uses

Characteristic	Controls			MDMA polydrug users		
	Mean	SD	Range	Mean	SD	Range
Alcohol intoxication	179	459	0-2000	269	219	5-728
Cannabis, lifetime	13	18	0-65	827	945	10-3500
Amphetamine	0	n/a	0	129	281	0-1500
Cocaine	0.05	0.24	0-1	226	756	0-3150
Psilocybin	0.6	1	0-5	16	24	0-100
Ephedrine	0	n/a	0	140	325	0-1000
LSD	0	0	0-1	29	33	0-110
Tobacco	212	603	0-2300	7030	9579	0-30000

LSD= Lysergic acid diethylamide; n/a = not applicable.

Table 3 The regions in which MDMA polydrug users and controls differ in normalized ^{11}C -AMT trapping ($p < 0.005$; cluster threshold: ≥ 40)

MNI coordinates			Brain Region/Brodmann Area (BA)	# voxels	Peak T
X [mm]	Y [mm]	Z [mm]			
Normalized ¹¹ C-AMT trapping in MDMA polydrug users < controls:					
0	-20	60	L. Medial frontal gyrus; BA 6	432	4.09
0	-56	56	L. Precuneus; BA 7	340	3.62
48	-4	34	R. Precentral Gyrus; BA6	44	3.38
-34	-36	54	L. Postcentral Gyrus, Parietal Lobe	76	3.31
38	-60	44	Parietal lobe, R. Inferior Parietal Lobule; BA 40	126	3.29
22	-64	44	R. Precuneus; BA 7	126	3.15
44	28	-6	R. Inferior Frontal Gyrus; R. Inferior Orbitofrontal Gyrus	40	3.26

-38	34	-14	L. Middle Frontal Gyrus; R. Inferior Orbitofrontal Gyrus; BA11	65	3.10
-36	-6	50	L. Middle Frontal Gyrus; BA 6	42	3.09
Normalized ^{11}C -AMT trapping in MDMA polydrug users > controls:					
-2	-26	-20	L. Midbrain (Brain stem)	62	3.63
-20	44	46	L. Superior Frontal Gyrus	343	3.56
-26	36	50	L. Middle Frontal Gyrus	343	2.95
-32	48	32	L. Superior Frontal Gyrus; BA 9	343	2.63
-36	8	-28	L. Temporal Lobe; L. Superior Temporal Gyrus	55	3.35

L=left. R=right. BA= Brodmann area. MNI=Montreal Neurological Institute

Table 4 The regions in which male and female MDMA polydrug users have lower normalized ^{11}C -AMT trapping than controls ($p < 0.005$; cluster threshold: ≥ 40)

MNI coordinates			Brain Region	# voxels	Peak T
X [mm]	Y [mm]	Z [mm]			
Females: MDMA users < controls					
2	-60	50	R. Precuneus, BA7	44	3.41
-34	36	-18	L. Inferior Frontal Gyrus; L. Inferior Orbitofrontal cortex	60	3.30
6	-24	60	R. Medial Frontal Gyrus, BA 6	61	3.29
Males: MDMA users < controls					
-24	-8	-38	L. Limbic lobe, BA 28	384	3.66
-38	-10	-48	L. Inferior Temporal Gyrus	384	4.05
-6	-52	58	L. Precuneus	82	3.99
50	-2	32	R. Precentral Gyrus; BA6	57	3.97
46	24	-6	R. Inferior Frontal Gyrus; Inferior Orbitofrontal cortex	65	3.63

-28	-34	56	L. Postcentral Gyrus; BA 3	155	3.65
-32	-10	54	L. Precentral Gyrus; BA6	155	3.25
-32	-18	52	L. Precentral Gyrus	155	3.32

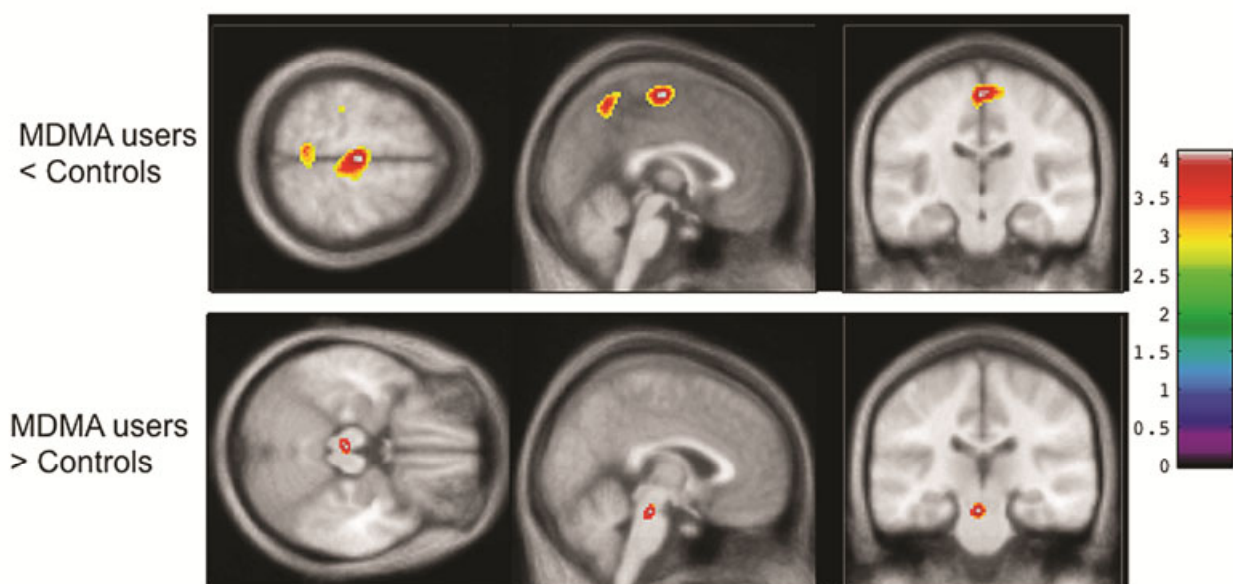
L=left. R=right. BA= Brodmann area. MNI=Montreal Neurological Institute

Table 5 Regional normalized K* (mean $\pm$ SD) in selected Volume of Interests (VOIs)

Brain region	MDMA polydrug users		Controls	
	Males	Females	Males	Females
Raphe	84.0(24.4)	94.7(14.1)	81.6 (23.3)	73.1(16)
Brain stem	92 (5.1)	91.9(3.6)	86.5(7.5)	84.5(17.8)
Precentral gyrus	105.9(4.8)	110.4(5.6)	112.8(7.1)	110 (15.3)
Postcentral gyrus	106.3(5.6)	109.9(3.4)	111(7.9)	103.5(11)
Precuneus	119.1(8.2)	118.1(11.7)	120.8(10.8)	118.7(5.9)
Med. frontal gyrus	117 (4.3)	119.5(4.7)	115.3(7.9)	122 (6.6)
Middle frontal gyrus	115.5(4.8)	116.6(6.0)	112.1(5.7)	107.7(13.4)
Superior frontal gyrus	103.6(10)	105.9(6.7)	99.7(16.3)	104.0(4.8)
Superior temporal gyrus	124.8(4.7)	124.5(5.6)	121.1(3.6)	118 (5.7)
Superior parietal lobule	113.1(7)	110.9(6.1)	113.4(10.5)	114.8(15.4)
Med. Orbitofrontal gyrus	129.4(5.6)	120.9(10.4)	117.7(18.4)	122.7(27.3)
Lat. Orbitofrontal gyrus	104.6(12.4)	89.3(8.8)	98.4(11.9)	100.8(11.2)

Note. Med.=medial; Lat.=lateral. For the brain stem, the difference between the MDMA polydrug user and control group was a trend. ($F(1,16)=3.27$, $p=0.089$, $d=0.85$).

Normalized ^{11}C -AMT trapping in MDMA polydrug users (n=18) vs. controls (n=17)



Severity / duration of use and normalized ^{11}C -AMT trapping in MDMA polydrug users

